REMARKS

Reconsideration and allowance are respectfully requested.

Claims 1, 5-14 and 17-20 are pending. Applicants acknowledge the Examiner's indication that claims 1, 5-13 and 17-19 are allowable.

Applicants thank the Examiner for the courtesy extended during the interview of August 10, 2005. At the interview, the Examiner suggested that Applicants submit evidence (if available) showing that there was not a reasonable expectation of success because there were reasons to doubt that expressing an enzyme having neoxanthin cleavage activity would increase stress tolerance. In response to this invitation, Applicants submit that uncertainties surrounding the determination of the steady-state level of ABA (both its biosynthesis and catabolism) and the mechanisms of stress tolerance in plants show that the premise of the obviousness rejection (i.e., there was a reasonable expectation that increasing ABA would increase a plant's stress tolerance) is incorrect. Evidence bearing on these uncertainties is discussed below.

35 U.S.C. 103 – Nonobviousness

A determination of *prima facie* obviousness requires a reasonable expectation of success. See *In re Rinehart*, 189 USPQ 143, 148 (C.C.P.A. 1976).

Claims 14 and 20 were rejected under Section 103(a) as allegedly unpatentable over Wu *et al.* in view of Tan *et al.* and Swamy *et al.* Applicants respectfully traverse.

The Examiner has maintained that it would have been *prima facie* obvious to make stress tolerant plants by substituting DNA encoding maize VP14 protein for DNA encoding LEA protein in the method of Wu *et al.*, because there would have been a reasonable expectation of success, based on the effects of exogenous ABA, that an increase in a plant's production of endogenous ABA would increase stress tolerance. But the prior art teaches, on the contrary, that a plant's increased expression of neoxanthin cleavage enzyme (*i.e.*, 9-*cis* epoxycarotenoid dioxygenase, NCED) would not necessarily have that effect and, therefore, a reasonable expectation of success was lacking at the time the claimed invention was made.

Based on what was known about ABA synthesis in the prior art, one of ordinary skill in the art would not have had a reasonable expectation of success over-expressing NCED to increase the amount of endogenous ABA. First, the biosynthetic pathway of ABA *in planta* was known to be complex and involved enzyme-catalyzed steps subsequent to neoxanthin cleavage. Thus, even if NCED over-expression increased its cleavage product xanthoxin, improper functioning of any of these downstream steps would prevent the increase in the product ABA. Second, ABA catabolism *in planta* was known to be important in determining the level of endogenous ABA and its antagonistic effect could have prevented newly synthesized ABA from accumulating. These points are discussed in more detail below.

I. ABA Biosynthesis

Neoxanthin cleavage enzyme (NCED) catalyzes the rate-limiting step in ABA biosynthesis. The complexity of the biosynthetic pathway is shown in Fig. 1 of Taylor *et al.* (J. Exp. Bot. 51:1563-1574, 2000; a copy of which is attached). NCED was thought to catalyze steps E and F. ABA-deficient mutants are listed in Table 1 of Taylor *et al.* After the NCED-catalyzed step, xanthoxin (XAN) is converted to ABA aldehyde (ABAld), then ABAld is converted to ABA. Dysfunction in either or both of these two steps would prevent synthesis of ABA even if NCED was over-expressed. Taylor *et al.* conclude on page 1573, "It is important to know how expression of the three genes [zeaxanthin epoxidase, NCED, and ABA aldehyde oxidase] is co-ordinated to ensure that ABA synthesis can be sustained efficiently." At the time the present invention was made, it would have been naïve to have believed that simply over-expressing NCED *in planta* would result in an increase in endogenous ABA, because there was a possibility that the improper functioning of other biosynthetic enzymes might prevent production of ABA.

Therefore, one of ordinary skill in the art would not have had a reasonable expectation of success that over-expressing NCED would result in increased ABA production as found by Applicants until the appropriate plants were engineered and analyzed.

II. ABA Catabolism

Knowledge of the catabolic pathway of ABA was incomplete, but 8' hydroxylase was thought to play a predominant role. Windsor *et al.* (Phytochem. 45:931-934, 1997; a copy of which is attached) state on page 931,

> "The active concentrations of ABA in plant tissues is determined by the rates of its biosynthesis and catabolism. The major pathway of inactivation of ABA commences with hydroxylation (Fig. 1), yielding 8'-hydroxy-ANA which is unstable and rearranges to phaseic acid (PA). [citations deleted]"

Similarly, Kushiro *et al.* (EMBO J. 23:1647-1656, 2004; a copy of which is attached) state on page 1647,

> "ABA content in plants is determined by the balance of between its biosynthesis and catabolism. When high levels of ABA are maintained, both ABA biosynthesis and catabolism are active."

See also, Fig. 1 of Kushiro *et al.* Exogenous ABA increased the expression of the 8' hydroxylase. Table 1 of Windsor *et al.* and Fig. 7B of Kushiro *et al.* It was thought that stimulation with exogenous ABA triggered catabolism to regulate the final amount of endogenous ABA. Thus, increased production of ABA could be counter-balanced by its catabolism. Kushiro *et al.* at page 1653 ("it has been argued that ABA levels are maintained by the balance between its biosynthesis and catabolism, rather than simply by biosynthesis alone"). At the time the present invention was made, it would have been naïve to have believed that merely over-expressing NCED would result in accumulation of ABA, because it might be catabolized by 8' hydroxylase as quickly as it was produced.

Therefore, one of ordinary skill in the art would not have had a reasonable expectation of success that over-expressing NCED would result in increased ABA production as found by Applicants until the appropriate plants were engineered and analyzed.

As discussed above, the amount of endogenous ABA *in planta* results from the interplay between biosynthetic and catabolic pathways. Multiple enzymes are involved. In addition, the possibility of feedback inhibition by ABA adds another layer of complication. Taylor *et al.* conclude their review on page 1573,

> "It is also necessary to remember that the balance between ABA synthesis and metabolism is vital in determining whether ABA accumulates in whole

> plants. ABA is metabolized by ABA 8'-hydroxylase activity which is induced by ABA itself. This effect is clearly antagonistic to attempts to increase ABA levels by over-expressing ABA biosynthesis genes If ABA were also to inhibit its own synthesis by some form of feedback, then a combination of these two effects would tend to frustrate attempts to produce transgenic plants with elevated ABA levels. [citations deleted]"

Although published after the effective priority of this application, the Taylor *et al.* document provides evidence of the lack of a reasonable expectation of success at a period of time contemporaneous to Applicants' making of the claimed invention.

Withdrawal of the Section 103 rejection is requested because the invention as claimed would not have been obvious to a person of ordinary skill in the art at the time it was made.

Conclusion

Having fully responded to all of the pending objections and rejections contained in this Office Action, Applicants submit that the claims are in condition for allowance and earnestly solicit an early Notice to that effect. The Examiner is invited to contact the undersigned if any further information or discussion is required.

Respectfully submitted,

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